

## METHODS AND REAGENTS FOR REDUCING POLYGLUTAMINE TOXICITY

## 5 CROSS-REFERENCE TO RELATED APPLICATION(S)

This application claims the benefit of U.S. Provisional Application, Ser. No. 60/451,077, filed February 27, 2003, the contents of which is hereby incorporated by reference in its entirety.

## 10 BACKGROUND OF THE INVENTION

Huntington's disease (HD) is an autosomal dominant disorder which affects 1 in 10,000 individuals worldwide. It is caused by an expansion in a polyglutamine (polyQ) repeat in the amino-terminal domain of the protein, Huntingtin (Htt), a 350kD largely cytoplasmic protein of unknown function. HD is one of several neurodegenerative diseases including spinobulbar muscular atrophy, the spinocerebellar ataxias, and DRPLA, caused by polyQ expansions in otherwise unrelated proteins.

In HD and other polyQ diseases, mutant proteins and/or pathogenic polyQ peptides produced by proteolytic processing aggregate into nuclear and/or cytosolic inclusions in neurons and in neuronal processes. These aggregates also contain other cellular proteins including transcription regulating proteins, chaperones, proteasome subunits, and ubiquitin (G. Bates, Lancet 361, 1642-4 (2003); S. Steffan, L. M. Thompson, Expert Opin. Ther. Targets 7, 201-13 (2003); A. J. Tobin, E. R. Signer, Trends Cell. Biol. 10, 531-6 (2000)).

PolyQ disease proteins can be modified in ways that change their cellular function or fate. Htt is subject to ubiquitination, which normally targets proteins for degradation (S. W. Davies et al., Cell 90, 537-548 (1997); M.

A. Kalchman et al., J Biol Chem 271, 19385-94 (1996)).  
 Mutants in ubiquitin ligases enhance polyQ toxicity in  
 Drosophila, mouse and cell models (C. J. Cummings et al.,  
 Neuron 24, 879-92 (1999); P. Fernandez-Funez et al., Nature  
 408, 101-106 (2000); F. Saudou, S. Finkbeiner, D. Devys, M.  
 Greenberg, Cell 95, 55-66 (1998)) while over expression of  
 Parkin, an E3 ubiquitin ligase, can reduce polyQ aggregation  
 and suppress cytotoxicity (Y. C. Tsai, P. S. Fishman, N. V.  
 Thakor, G. A. Oyler, J Biol Chem 278, 22044-22055 (2003)).  
 Thus, ubiquitination appears to reduce polyQ toxicity  
 presumably by promoting degradation of toxic peptides.

SUMOylation is a post-translational modification system  
 [for review see (F. Melchior, Annu Rev Cell Dev Biol 16, 591-  
 626 (2000); S. Muller, C. Hoege, G. Pyrowolakis, S. Jentsch,  
 Nat Rev Mol Cell Biol 2, 202-10 (2001))] that is biochemically  
 similar to, but functionally distinct from ubiquitination. It  
 involves covalent attachment of SUMO-1 ("small ubiquitin-like  
 modifier") to lysine residues. SUMOylation can alter protein  
 function or subcellular location, and competition between  
 SUMO-1 and ubiquitin for identical target lysines can protect  
 some proteins from degradation (J. M. Desterro, M. S.  
 Rodriguez, R. T. Hay, Mol Cell 2, 233-9 (1998); C. Hoege, B.  
 Pfander, G. L. Moldovan, G. Pyrowolakis, S. Jentsch, Nature  
 419, 135-41 (2002); X. Lin, M. Liang, Y. Y. Liang, F. C.  
 Brunnicardi, X. H. Feng, J Biol Chem 278, 31043-31048 (2003)).  
 In addition, the majority of SUMO-modified proteins found in  
 the cell are located in the nucleus (A. Pichler, F. Melchior,  
 Traffic 3, 381-7 (2002)) and SUMOylation can have a direct  
 effect on nucleocytoplasmic transport.

Here we investigate the role of SUMOylation in HD  
 pathogenesis. In this study we have found that Huntingtin is

modified by SUMO-1 and co-localizes with PML and SUMO-1 in cell culture and in transgenic mouse brain. In addition, we have found that SUMO-1 modification of PML is reduced in transgenic mouse brain. Huntingtin is stabilized by SUMO-1 modification, as the same lysine residues which appear SUMOylated can also be ubiquitinated. In *Drosophila*, we have shown that a reduction in cellular SUMOylation results in a rescue of photoreceptor neuron degeneration induced by Huntingtin. Therefore, drugs which reduce SUMO-1 modification of Huntingtin, or increase cleavage of SUMO-1 from Huntingtin, may be useful in the treatment of HD.

#### BRIEF SUMMARY OF THE INVENTION

Expression of a truncated portion of the mutant Huntingtin protein encoded by exon 1 of the *HD* gene (Httex1p) causes disease similar to Huntington's disease (HD) in transgenic mice and flies. Httex1p can be modified by SUMO-1 and ubiquitin on lysines 6, 9, and 15; mutagenesis of these lysines to arginines reduces stability of the protein. Crossing a *Drosophila* model of HD with a reduced function *smt3* (*Drosophila* SUMO) mutant results in suppression of lethality and neurodegeneration. Therefore, a drug therapy designed to lower SUMO-1 modification in the cell, or increase cleavage of SUMO-1 from target proteins, should be useful to destabilize Httex1p and block neurodegeneration in HD and other polyglutamine repeat diseases such as Kennedy's disease, dentatorubral-pallidoluysian atrophy, spinocerebellar ataxia, types 1, 2, 3 (Machado-Joseph), 6 and 7, TBP (severe cerebellar atrophy), and others, as well as other neurological diseases and disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, epilepsy,

diabetes mellitus, spongiform encephalopathy, prion-related disease, and schizophrenia..

5 Thus, one embodiment of the present invention provides a method of treating neurodegeneration in a patient, comprising identifying a patient at risk for neurodegeneration; and administering to the patient a therapeutically effective amount of SUMOylation blocker.

10 Another embodiment of the present invention provides a method of treating neurodegeneration in a patient, comprising identifying a patient at risk for neurodegeneration; and administering to the patient a therapeutically effective amount of deSUMOylation enhancer.

15 Another embodiment of the present invention provides a method of treating neurodegeneration in a patient, comprising identifying a patient at risk for neurodegeneration; and administering to the patient a therapeutically effective amount of a Ubiquitination activator.

20 Another embodiment of the present invention provides a method of treating polyglutamine-expansion-related neurodegeneration in a patient, comprising identifying a patient at risk for polyglutamine-expansion-related neurodegeneration; and administering to the patient a therapeutically effective amount of SUMOylation blocker.

25 Another embodiment of the present invention provides a method of treating polyglutamine-expansion-related neurodegeneration in a patient, comprising identifying a patient at risk for polyglutamine-expansion-related neurodegeneration; and administering to the patient a therapeutically effective amount of deSUMOylation enhancer.

30 Another embodiment of the present invention provides a method of treating polyglutamine-expansion-related

neurodegeneration in a patient, comprising identifying a patient at risk for polyglutamine-expansion-related neurodegeneration; and administering to the patient a therapeutically effective amount of a Ubiquitination activator.

Another embodiment of the present invention provides a method of treating a neurodegenerative disease in a patient, comprising administering to the patient a therapeutically effective amount of SUMOylation blocker.

Another embodiment of the present invention provides a method of treating a neurodegenerative disease in a patient, comprising administering to the patient a therapeutically effective amount of deSUMOylation enhancer.

Another embodiment of the present invention provides a method of treating a neurodegenerative disease in a patient, comprising administering to the patient a therapeutically effective amount of a Ubiquitination activator.

Another embodiment of the present invention provides a method of treating Huntington's disease in a patient, comprising administering to the patient a therapeutically effective amount of a SUMOylation blocker.

Another embodiment of the present invention provides a method of treating Huntington's disease in a patient, comprising administering to the patient a therapeutically effective amount of a deSUMOylation enhancer.

Another embodiment of the present invention provides a method of treating Huntington's disease in a patient, comprising administering to the patient a therapeutically effective amount of a Ubiquitination activator.

Another embodiment of the present invention provides a method of treating Kennedy's disease in a patient, comprising

administering to the patient a therapeutically effective amount of a SUMOylation blocker.

5 Another embodiment of the present invention provides a method of treating Kennedy's disease in a patient, comprising administering to the patient a therapeutically effective amount of a deSUMOylation enhancer.

10 Another embodiment of the present invention provides a method of treating Kennedy's disease in a patient, comprising administering to the patient a therapeutically effective amount of a Ubiquitination activator.

15 Another embodiment of the present invention provides a method of treating spinocerebellar ataxia in a patient, comprising administering to the patient a therapeutically effective amount of a SUMOylation blocker.

20 Another embodiment of the present invention provides a method of treating spinocerebellar ataxia in a patient, comprising administering to the patient a therapeutically effective amount of a deSUMOylation enhancer.

25 Another embodiment of the present invention provides a method of treating spinocerebellar ataxia in a patient, comprising administering to the patient a therapeutically effective amount of a Ubiquitination activator.

30 Another embodiment of the present invention provides a method of treating dentatorubral-pallidoluysian atrophy in a patient, comprising administering to the patient a therapeutically effective amount of a SUMOylation blocker.

35 Another embodiment of the present invention provides a method of treating dentatorubral-pallidoluysian atrophy in a patient, comprising administering to the patient a therapeutically effective amount of a deSUMOylation enhancer.

Another embodiment of the present invention provides a

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method of treating dentatorubral-pallidoluysian atrophy in a patient, comprising administering to the patient a  
5 therapeutically effective amount of a Ubiquitination activator.

Another embodiment of the present invention provides a method of treating protein-aggregation-related  
10 neurodegeneration in a patient, comprising administering to the patient a therapeutically effective amount of a SUMOylation blocker.

Another embodiment of the present invention provides a method of treating protein-aggregation-related  
15 neurodegeneration in a patient, comprising administering to the patient a therapeutically effective amount of a deSUMOylation enhancer.

Another embodiment of the present invention provides a method of treating protein-aggregation-related  
20 neurodegeneration in a patient, comprising administering to the patient a therapeutically effective amount of a Ubiquitination activator.

Another embodiment of the present invention provides a  
25 method of treating Machado-Joseph's disease in a patient, comprising administering to the patient a therapeutically effective amount of a SUMOylation blocker.

Another embodiment of the present invention provides a  
30 method of treating Machado-Joseph's disease in a patient, comprising administering to the patient a therapeutically effective amount of a deSUMOylation enhancer.

Another embodiment of the present invention provides a  
35 method of treating Machado-Joseph's disease in a patient, comprising administering to the patient a therapeutically effective amount of a Ubiquitination activator.

BRIEF DESCRIPTION OF THE DRAWINGS

**FIG. 1.** Huntingtin can be modified by SUMO-1 or Ubiquitin and co-localizes with SUMO-1 in cell culture. (A) The sequence of the Httexlp fragment with the extent of the two transgene constructs indicated. (B) SUMO-1 co-localizes with expanded Httexlp at the nuclear periphery and in inclusions of immortalized striatal neurons (cytoplasm not visible due to fixation technique used). (C) Httexlp with and without the proline-rich region is modified by HIS-SUMO-1 or by HIS-Ubiquitin in HeLa cells. Cells were co-transfected with plasmids expressing HIS-tagged SUMO-1 or ubiquitin, and Httexlp 97QP or 103Q. The tagged protein was enriched using Ni-NTA magnetic nickel columns and detected by Western analysis using anti-Htt antibody. Mutation of all lysine residues [K] to arginine [R] (K6,9,15R) inhibits both SUMOylation and ubiquitination of Httexlp. 10% of the initial Ni-NTA lysate was TCA precipitated and subjected to Western analysis, showing relative levels of modified and unmodified Httexlp (WC-TCA). (D) Unexpanded 25QP Httexlp can be modified by SUMO-1 and ubiquitin. 97QP-GFP, 103Q-GFP, and 25QP-GFP with wt lysines 6, 9, and 15 (control) or with these lysines mutated to arginine (K6,9,15R) were transiently co-transfected with vector control, HIS-SUMO-1, or HIS-Ubiquitin. Ni-NTA enrichment was performed as in C. Unique SUMO-1 and ubiquitin-modified bands, present in the control, but not in the K6,9,15R mutants are denoted by black circles. (E) Mutation of the lysine residues singly and in combination significantly reduced or eliminated Httexlp modification by HIS-SUMO-1 or HIS-Ubiquitin in HeLa cells as demonstrated by Ni-NTA enrichment and Western analysis using anti-Htt antibodies.



**FIG. 2.** Huntingtin is stabilized by SUMO-1 modification and fusion of SUMO-1 to Httex1p or deletion of the proline-rich region of Httex1p decreases inclusion formation. (A) The abundance of untagged 97QP or 103Q Httex1p is reduced by mutation of amino-terminal lysines to arginines. HeLa cells were co-transfected with plasmids expressing modified Httex1p constructs and exogenous HIS-SUMO-1. Whole cell extracts were assessed by Western analysis using anti-Htt antibody. (B) Western analysis with anti-Htt antibody of SUMO-1 fused in frame with the K6,9,15R triple mutants of 97QP or 103Q in immortalized striatal cell extract demonstrates that "permanent SUMOylation" dramatically increases protein levels. Arrows indicate the unmodified size of the Htt transgenes, 97QP and 103Q. (C) Immortalized striatal cells were transiently transfected with a plasmid containing the CMV promoter fused to the gene for  $\beta$ -galactosidase (pCMX- $\beta$ gal) along with either pCDNA3.1 vector control, 97QP, 97QP K6,9,15R, SUMO-97QP, or SUMO-97QP K6,9,15R. SUMO-97QP dramatically represses transcription of the CMV promoter. (D) Immunofluorescence analysis shows that while inclusions are observed for 97QP and its triple mutant in immortalized striatal cells, inclusion formation is reduced by SUMO-1 fusion to the 97QP and 97QP K6,9,15R. Fusion of SUMO-1 to Htt 97QP produces diffuse staining predominantly in the cytoplasm in cells where expression is visible. (E) Immunofluorescence shows that inclusions are found in immortalized striatal cells expressing 97QP but not 103Q, demonstrating a role for the proline-rich region of Httex1p in aggregation.

**FIG. 3.** Permanently SUMOylated Httex1p dramatically represses transcription. Luciferase assays were performed using immortalized striatal cells transiently co-transfected

with either the MDR1-luciferase or WAF1-pGL3-luciferase reporters and with pcDNA3.1 vector control, 97QP, or SUMO permanently fused to 97QP (SUMO-97QP).

**FIG. 4.** Amino acids 1-17 of Htt can target a nuclear-localized reporter protein to the cytoplasm, independent of CRM-1 function. Leptomycin B abolishes nuclear export that is mediated by the CRM-1 export receptor. NIH-3T3 cells were transfected with the indicated GFP reporter constructs. After 24 hours cells were treated with Leptomycin B for 2 hours (+LMB) or not (Mock) and GFP localization was recorded by confocal microscopy. Single sections are shown. GFP alone locates to both cytoplasm and nucleus. When fused to a NLS, GFP is nuclear. Fusion of the first 17 amino acids of Htt to the NLS-GFP construct drives GFP to the cytoplasm and this localization is unaffected by LMB. Control experiments demonstrate that CRM dependent nuclear export signals, NES-GFP, are quite sensitive to LMB.

**FIG. 5.** Genetic reduction of SUMO activity in *Drosophila* reduces neurodegeneration in an HD fly model. (A) Neuropathology improves when SUMO levels are reduced. Flies expressing Httex1p Q93 ubiquitously in the nervous system under the control of the elav-GAL4 driver show extensive loss of photoreceptors (gray bars). Normal flies show 7 rhabdomeres in every ommatidium and the more extensive the degeneration, the fewer the number of rhabdomeres. When the level of SUMO activity is reduced by 50% in heterozyotes of the single SUMO gene in *Drosophila* (smt3/+), photoreceptor loss is dramatically reduced (black bars); thus, genetic reduction of SUMO activity rescues HttQ93 mediated neuropathology. T-test of significance =  $P < 0.001$ . (B) Neuropathology is only modestly increased when ubiquitin

activity is reduced. When the level of ubiquitin activity is reduced by 50% in heterozygotes of the Ubi63E ubiquitin gene (Ubi63E/+), photoreceptor loss is modestly more severe (black bars) than in controls expressing Htt Q93 in a normal background. Indeed the increase in severity is barely significant statistically  $P < 0.060$ . Thus, genetic reduction of ubiquitin activity only slightly exacerbates HttQ93 mediated neuropathology. (C) Cytotoxicity is severely reduced by mutation of the three lysines in Htt 97QP. Transgenic Drosophila expressing Httex1p 97QP or Httex1p 97QP K6,9,15R under the control of elav-GAL4 at 27° were analyzed. Expression of the unmodified 97QP transgene produces significant photoreceptor loss (black bars). In contrast, when the transgene with the three lysines mutated is expressed, photoreceptor loss is dramatically reduced [ $P < 0.013$ ] indicating a strong attenuation of cytotoxicity by mutation of these lysines. D. Transgenic Drosophila expressing Httex1p 97QP or Httex1p 97QP K6,9,15R under the control of gmr-GAL4 at 27° were analyzed. Expression of the unmodified Httex1p 97QP transgene produces a visible rough eye phenotype with necrotic lesions indicative of cytotoxicity. In contrast, expression of the transgene with the three lysines mutated produces almost no detectable phenotype under the same conditions again confirming a strong attenuation of cytotoxicity by mutation of these lysines.

#### DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices and materials similar

or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing, for example, the cell lines, constructs, and methodologies that are described in the publications which might be used in connection with the presently described invention. The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

Long repeats of polyglutamines within specific disease genes are responsible for at least eight human neurodegenerative diseases, including Huntington's disease (HD). Expression of a truncated portion of the mutant Huntingtin protein encoded by exon 1 of the *HD* gene (*Httex1p*) causes neurodegenerative disease similar to HD in transgenic mice and flies. SUMO-1 (small ubiquitin-related modifier-1) modification of proteins affects their stability, protein-protein interactions, and/or subcellular localization. *Httex1p* was found to be SUMO-1 modified and ubiquitinated. Mutation of three lysine residues in the amino-terminal 17 amino acids of expanded *Httex1p* to arginine (K6R, K9R, and K15R) reduces stability of this polypeptide in cell culture. In mutagenesis studies, lysines 6, 9, and 15 were found to be important for SUMOylation of *Httex1p* and ubiquitin and SUMO-1 may compete for lysines 6 and 9. PML SUMOylation is reduced in HD transgenic mouse brain. Since in polyQ disease brains, nuclear body morphology is changed, a reduction in SUMOylation

of PML may be instrumental in the disruption of nuclear body structure. Expanded polyQ Httexlp co-localizes with PML and SUMO-1 in nuclear bodies in transgenic mouse brain and in cell culture, consistent with many nuclear body proteins found to be modified by SUMO-1. Crossing a *Drosophila* model of HD with a reduced function *smt3* (*Drosophila* SUMO) mutant results in suppression of lethality and of neurodegeneration of photoreceptor neurons in the eye. Therefore a drug therapy designed to lower SUMOylation in the cell, or increase cleavage of SUMO-1 from target proteins such as Htt, should be useful to destabilize Httexlp and block neurodegeneration in HD and other polyQ diseases.

There are currently no tested compounds that rescue the neurodegeneration or prevent progression of disease in HD patients. Blockage of the process of SUMOylation of proteins, or enhancement of the process of deSUMOylation of proteins, may prevent neurodegeneration and death caused by polyglutamine repeat diseases and other diseases caused by aberrant aggregation of protein. To our knowledge, drugs which can accomplish either of these processes have not been developed and are not available. Drugs that are able to block SUMOylation (hereinafter, "SUMOylation blocker") or enhance deSUMOylation (hereinafter, "deSUMOylation enhancer") should be useful in treatment of neurodegenerative disease. Potential therapeutic drugs include agents that decrease the activities of E1 SUMO activating enzyme, E2 SUMO conjugating enzyme, or E3 SUMO ligating enzyme, or which increase the activity of SUMO isopeptidase, as well as any other agent shown to be effective in reducing SUMOylation of Huntingtin and other poly-Q repeat proteins. PIAS proteins, a class of SUMO E3 ligase enzymes (a protein inhibitor of activated STAT)

SUMOylate Huntingtin (data not shown) and inhibition of the function of these PIAS proteins in particular may represent a good therapeutic agent for treatment of neurodegenerative diseases. Other potential therapeutic agents include drugs designed to enhance the process of Ubiquitination (activate E1 Ubiquitin activating enzyme, activate E2 Ubiquitin conjugating enzyme, activate E3 Ubiquitin ligating enzymes; hereinafter, "Ubiquitination activators") or to inhibit deUbiquitination (such as inhibitors of Ubiquitin isopeptidase, to decrease cleavage of Ubiquitin from proteins; hereinafter "deUbiquitination inhibitors").

The present inventors have provided a clear demonstration of reduced polyglutamine toxicity in response to a loss in the ability of *Drosophila* cells to sumoylate proteins *in vivo*. It is proposed that a reduction in the SUMOylation of pathogenic mutant polyglutamine repeat protein causes it to become unstable and to be degraded much more quickly than when it is SUMOylated. Therefore, drugs designed to block SUMOylation of mutant polyglutamine repeat proteins, or drugs designed to enhance the removal of SUMO-1 from these proteins, should have a therapeutic effect in the treatment of HD and other polyglutamine-repeat diseases.

The consequence of polyglutamine repeat disease is slow and wasting death with no treatment options available. Any option to slow or prevent the process would be desirable. The invention has clear public and commercial use in the treatment of HD and other polyglutamine repeat diseases and potentially as well in neurodegenerative and psychiatric diseases in general.

# **MATERIALS AND METHODS**

**Plasmid constructs.** Httex1p-GFP fusion proteins were  
 5 created by placing alternating CAG/CAA repeats, coding for  
 either a normal range or expanded polyglutamine tract, into  
 the context of either a truncated [first 17 amino acids plus  
 poly(Q) repeat] or complete Huntingtin exon 1 containing the  
 10 proline-rich region and subcloning into pcDNA 3.1. Untagged  
 Httex1p constructs were created from these GFP-tagged  
 constructs by blunting the BamHI and XbaI sites surrounding  
 the GFP cDNA and reclosing the vector. K6R, K9R, and K15R  
 mutations in Httex1p were created through use of double-  
 15 stranded oligonucleotides containing HindIII compatible ends,  
 encoding the first 17 amino acids of Huntingtin (plus and  
 minus lysine to arginine mutations in amino acids 6, 9, and  
 15), which were ligated between the HindIII site of pcDNA3.1  
 20 in the polylinker, and the HindIII site in exon I, immediately  
 5' to the CAG repeat. Double-stranded oligonucleotides were  
 used to fuse the SV40 nuclear localization signal (MGPKKKRK)  
 to the amino-terminus of EGFP, and Htt amino acids 1-17 were  
 then fused to the amino-terminus of NLS-EGFP to create both  
 25 NLS-EGFP and 1-17 NLS-EGFP constructs. pEGFP-N1 (BD  
 Biosciences/Clontech) was used as a control plasmid for EGFP  
 expression. pCMX-betagal (containing the CMV promoter fused to  
 the beta-galactosidase gene) was the gift of B. Blumberg  
 30 (UCI)/ M. Tabb (UCI)/R. Evans (Salk Institute). A PCR  
 fragment encoding amino acids 1-96 of SUMO-1 (lacking glycine  
 97 creating a SUMO-1 that is not susceptible to proteolytic  
 removal by isopeptidases) was fused in frame to the amino-  
 terminus of 97QP, 97QP K6,9,15R or 103Q K6,9,15R with  
 35 SalI/NcoI linkers, creating "permanently" SUMOylated Httex1p  
 in pcDNA3.1. pHA-SUMO-1, pHIS-SUMO-1, and pHIS-Ubiquitin were

obtained from M. Nevels (Princeton), A. Dejean (Institut Pasteur)/G. David (Harvard)/R. DePinho (Harvard), and D. Bohmann (University of Rochester)/G. David (Harvard)/R. DePinho (Harvard), respectively. Synthetic oligonucleotides were used to create NES-GFP as a derivative of C2-EGFP from Clontech with the NES of PKI fused in frame at the C-terminus (GFP-NESPKI). 97QP and 97QP K6,9,15R KpnI/BamHI fragments were cloned between the EcoRI/NotI sites of pUAST to create pUAST-97QP and pUAST-97QP K6,9,15R. The proteins encoded by these constructs contain full Htt exon 1 including the DNA encoding the proline-rich region, followed by the following amino acid sequence: GSTSSRAAAARGYL. The MDR1-luciferase (a kind gift of E. Stanbridge, UCI) and WAF1-pGL3-luciferase reporter constructs were used as previously described (30).

**Assays.** Luciferase assays were conducted as previously reported (J. S. Steffan et al., Proc Natl Acad Sci U S A 97, 6763-8 (2000)). Magnetic nickel column Ni-NTA assays for SUMOylation and Ubiquitination were performed as previously described (S. Muller et al., J Biol Chem 275, 13321-9 (2000)). Beta-galactosidase activity was measured using the technique of Miller (J. H. Miller, Experiments in molecular-genetics, 352-355 (1972)). Briefly, 6 well dishes of HeLa cells were each transiently transfected with 100ng pCMX-betagal plasmid plus/minus 100ng test plasmid two days before harvest. The cells were lysed after a PBS wash in 200 microliters of Pharmingen 1X lysis buffer, spun in the microfuge for 30 seconds to remove debris, protein concentrations determined by Bradford assays. Beta-galactosidase activity is reported as % activity/mg protein.

Western analysis to examine levels of Huntingtin expression was done using whole cell lysates from HeLa or



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immortalized striatal cells broken and sonicated in Buffer A:  
10mM Tris-HCl pH 7.6, 1mM EDTA, 400mM NaCl, 10% glycerol, 0.5%  
5 NP-40, protease inhibitors: PMSF (1mM), Aprotinin (10  
micrograms/ml), Leupeptin (10 micrograms/ml), Iodoacetamide (2  
mM), and N-ethyl maleimide (20mM). Rabbit polyclonal Anti-Htt  
antibody CAG 53b was obtained from Erich Wanker.

10 The Leptomycin B Nuclear Export Signal Assay used NIH-3T3  
cells grown on glass coverslips (Fisher) and transfected using  
Lipofectamine 2000 according to the manufacturers protocol.  
Cells 24 hrs post-transfection were treated with 200 nM  
Leptomycin B (Sigma) or DMSO (Mock) for 2 hrs. Cells were  
15 fixed with 3% PFA and mounted using ProLong (Molecular Probes)  
mounting medium. Confocal microscopy was performed on a Zeiss  
LSM 510 confocal system using an Axiovert 100M microscope and  
a Plan/Apochromat 100x/Oil objective.

20 ***Drosophila* genetics.** *Elav>Httexon1Q93* animals were  
generated by crossing *elav-GAL4*; *Sb/TM6* virgins to UAS-  
exon1Q93 (line#P463) homozygous males at 18°C. Exon1Q93  
expressing males were then crossed to  $P(ry^{+t7.2=PZ})smt^{304493}$   
 $cn^1/CyO$ ;  $ry^{506}$  (Bloomington stock number 11378) females at 25°C,  
25 or to *Df(3L)HR119/TM6,Hu ca*, a deficiency that deletes the  
*Ubi63E* ubiquitin gene, (Stock #3649) and the *elav>exon1Q93*;  
*smt3/+* or *Ubi63E/+* progeny collected. Control animals were the  
*elav>exon1Q93*; *CyO* siblings. The eyes were scored using the  
30 pseudopupil technique (J. S. Steffan et al., Nature 413, 739-  
43 (2001)) at seven days post-eclosion. All crosses were  
performed using standard *Drosophila* medium.

Transgenic flies containing *uas-Httex1p 97QP* and *uas-*  
35 *Httex1p 97QP K6,9,15R* were generated by p-element  
transformation and confirmed by DNA sequencing. These  
constructs were crossed to flies expressing the yeast Gal4

transcriptional activator driven by either the *gmr* promoter which drives expression in all cells of the eye including neurons and surrounding and supporting cells ( $w^*$ ;  $P[w+mC=GAL4-ninaE.GMR]12$ ) (M. C. Ellis, E. M. O'Neill, G. M. Rubin, Development 119, 855-65 (1993)) or the *elav* promoter, which drives expression in all neurons from embryogenesis on  $pP[GAL4-elav.L]$ . Since the UAS/GAL4 system is highly sensitive to temperature, cultures were grown at 25°, 27° and 29°C.

**Cell culture and transfections.** HeLa cells were grown in Dulbecco's modified Eagle's medium (Invitrogen) with 10% fetal bovine serum (Invitrogen). ST12.7 rat striatal cells (S. Sipione et al., Hum Mol Genet 11, 1953-65 (2002)) were grown in Dulbecco's modified Eagle's medium (Invitrogen) with 10% fetal bovine serum (Invitrogen) at 33°C. HeLa cells were transfected with Effectene (QIAGEN) and rat striatal cells were transfected with FuGENE 6 (Roche) according to the manufacturer's instructions.

**Primary Antibodies.** The source and working dilution of primary antibodies were follows: S830 (1:500) is a sheep polyclonal raised against a GST-exon 1 huntingtin fusion protein carrying 53Q (a kind gift from Dr. G. Bates, London United Kingdom (D. L. Smith et al., Neurobiol Dis 8, 1017-26 (2001))). CAG53b (1:5000) is a rabbit polyclonal (raised against amino acids 1-118 with 51 polyglutamines; a kind gift from Dr. E. Wanker, Berlin, Germany) anti-huntingtin antibody (S. W. Davies et al., Cell 90, 537-548 (1997)). mEM48 (1:200) is a mouse monoclonal that was raised against a GST fusion protein containing the first 256 amino acids of human huntingtin with the deletion of the polyglutamine tract (Chemicon). SUMO-1 (FL-101) (1:500) is a rabbit polyclonal and

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SUMO-1 (D-11)(1:200) is a mouse monoclonal, both raised against human SUMO-1 (amino acids 1-101)(Santa Cruz  
5 Biotechnology, Inc).

**Immunofluorescence Analysis.** For experiments demonstrating co-localization of Htt with SUMO-1, immortalized striatal cells were permeabilized in 0.05% digitonin (Sigma) in PBS on ice for 5 min and washed in ice-cold PBS before  
10 fixation with 4% paraformaldehyde for 10 min and methanol for 2 min. The coverslips were incubated with the primary antibody followed by the Texas red (TR) and fluorescein (FITC) conjugated secondary antibodies. FITC conjugated anti-sheep  
15 (1:5000), FITC conjugated anti-rabbit (1:5000) and TR conjugated anti-mouse (1:1000) was from Jackson ImmunoResearch Laboratories. Next, the cells were incubated with DAPI and mounted in Vectashield (Vector Laboratories Inc). The cells were analyzed with Zeiss Axiovert 25 inverted microscope using  
20 AxioVision 3.0 imaging system (Carl Zeiss). Immunofluorescence (Figure 1B) to demonstrate co-localization of Httex1p and SUMO-1 was done using mouse anti-Htt EM48 antibody (Chemicon MAB 5374) and rabbit anti-SUMO-1 antibody  
25 (Santa Cruz Biotechnology FL-101). Immunofluorescence analysis in Figure 2D was done with S830 sheep anti-Htt antibody (1:500) and FITC secondary antibody. Immunofluorescence analysis in Figure 2E was done with anti-Htt CAG53b antibody (1:5000) and Texas Red secondary antibody.  
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**Unexpanded Httex1p is SUMOylated.** The first exon of Htt encodes only 3 lysine residues: K6, K9, and K15 (Fig. 1A). None of these lysines are within a precise ΨKXE consensus SUMOylation target sequence, Ψ being a hydrophobic residue and  
35 X any residue (A. Verger, J. Perdomo, M. Crossley, EMBO Rep 4, 137-42 (2003)), however there is precedence for non-consensus

SUMOylation sites [e.g. K65 of PML (T. Kamitani et al., J Biol Chem 273, 26675-82 (1998)) and PCNA (C. Hoege, B. Pfander, G. L. Moldovan, G. Pyrowolakis, S. Jentsch, Nature 419, 135-41 (2002))]. To determine if mutant Htt, previously shown to be ubiquitinated, can also be SUMOylated, we expressed HIS-SUMO-1 or HIS-ubiquitin in HeLa cells that were co-transfected with plasmids expressing expanded Httex1p either unmodified or with the 3 lysines mutated to arginine (K6,9,15R). We used magnetic nickel columns under denaturing conditions (Ni-NTA, Qiagen) to purify proteins covalently linked to HIS-SUMO-1 or HIS-ubiquitin followed by Western analysis with anti-Htt antibody to test for SUMOylation and ubiquitination of Httex1p. To eliminate any potential complications from SUMOylation of protein tags, no epitope tag was used in these experiments. We found 97QP and 103Q could both be SUMOylated and ubiquitinated, dependent on the presence of lysines 6, 9, and 15 (FIG. 1C and 1E). However, since the sensitivity of detection is greater when proteins are epitope-tagged, the experiments were also performed using polyQ Httex1p-GFP fusion proteins. Consistent with above results, higher molecular weight proteins corresponding to SUMOylated or ubiquitinated forms of both expanded and unexpanded polyglutamine versions of these fusions are observed in the presence of exogenous HIS-SUMO-1 or HIS-ubiquitin (FIG. 1D). This experiment demonstrates that unexpanded polyQ-repeat Httex1p (25QP-GFP) can be SUMO-1 modified as well as expanded polyQ 97QP-GFP and 103Q-GFP; we were unable to show this with untagged 25QP due to its low abundance in cellular extracts.

**Huntingtin can be modified by SUMO-1 or Ubiquitin and co-localizes with SUMO-1 in cell culture.** Truncated Htt (Httex1p 97QP, FIG 1A) and HIS-SUMO-1 co-localize when transfected into

immortalized striatal nerve cells, 12.7 (FIG 1B), reflecting either direct modification of Htt by SUMO-1 or colocalization of Htt with other SUMOylated proteins. To identify possible modifications of mutant Httex1p which contains only 3 lysine residues, K6, K9, and K15 (FIG 1A), HIS-SUMO-1 or HIS-ubiquitin was co-expressed with Httex1p either intact or with the lysines mutated to arginine (K6,9,15R) in both HeLa and striatal cells. We also compared Htt fragments either with or without the proline-rich domain immediately following the Qs, e.g. 97QP and 103Q respectively (FIG 1A). Both proteins can be SUMOylated or ubiquitinated (FIG 1C, lower panel) and a single primary SUMOylated species predominates, although more complex SUMO or ubiquitin modifications can be seen (FIG 1D). The low levels of Htt detected in the HIS-ubiquitin enriched fraction of cells transfected with the K6,9,15R triple mutant (FIG 1E) may reflect ubiquitination of the amino-terminus of the protein. The proline motif enhances SUMO-1 modification but suppresses ubiquitination, consistent with a possible influence of other proteins that interact with this region.

**Huntingtin is stabilized by SUMO-1 modification and fusion of SUMO-1 to Httex1p or deletion of the proline-rich region of Httex1p decreases inclusion formation.** All possible combinations of lysine mutants were generated within the 1st 17 amino acids of 97QP Httex1p, revealing that common residues are targeted for both SUMOylation and ubiquitination and specifically implicating residues K6 and K9 in these processes. When the lysine residues of Htt are mutated (double and triple lysine mutants) the abundance of Httex1p protein is reduced (FIG. 2A). Further, the proline-rich region of Httex1p also appears to contribute to the observed increase in soluble protein level since Htt peptides with the

prolines are more abundant than those without.

We explored 3 possible mechanisms whereby SUMO modification might influence pathology, namely aggregation, subcellular localization and transcriptional dysregulation. To isolate the effects of SUMO modification, we fused SUMO to the amino-terminus of Htt (S. Ross, et al., (2002) *Mol Cell* **10**, 831-42) and compared accumulation with and without the lysines and/or SUMO-1 fusion (FIG. 2B). The Htt peptide accumulates dramatically when fused to SUMO while the elimination of SUMOylation sites decreases levels.

Fusion of SUMO-1 to Httex1p also affects the aggregation properties of the protein. Using a dividing striatal neuronal cell line where nuclear localization is minimal, expression of Httex1p 97QP leads to the formation of large, Htt aggregates or inclusions (FIG. 2D; 97QP and 97QP K6,9,15R). Aggregate formation is also evident as Htt positive material that fails to penetrate the polyacrylamide gels of Western blots (FIG. 2B 97QP and 97QP K6,9,15R). However, when "permanently" SUMOylated, the levels of disperse cytoplasmic protein are increased and inclusions are reduced (FIG. 2D compare 97QP to SUMO-97QP, and 97QP K6,9,15R to SUMO-97QP K6,9,15R). In addition, aggregates of both SUMO-97QP and SUMO-97QP K6,9,15R are absent from the stacking gels of Western blots and soluble levels are increased (FIG. 2B). Thus, surprisingly, in addition to stabilizing the protein, SUMOylation appears to reduce the formation of visible, SDS-insoluble aggregates and increase disperse Htt staining in cells.

When the proline-rich domain is absent, (103Q) expression of Httex1p does not produce visible inclusions or SDS insoluble material (FIG. 2B,E), although when 103Q is fused to GFP numerous inclusions are evident, indicating that when not

fused to GFP, inclusion formation is dependent on the presence of the proline-rich region (FIG. 2E). We considered whether the apparent SUMO-induced increase in Htt stability might simply reflect a redistribution of Htt from inclusions to soluble material. However, because levels of 103Q also increase when permanently SUMOylated, even though Q103 does not form inclusions (FIG. 2B, E), this is not the case. We conclude that the proline-rich region of Httex1p is essential for inclusion formation and that SUMOylation of Httex1p both stabilizes the protein and reduces visible inclusion formation. It has been reported that ubiquitination of polyQ proteins can trigger formation of visible protein aggregates. If ubiquitination is essential for inclusion formation, then blocking the putative ubiquitination sites by SUMOylation might alter the protein aggregation state as observed. In any case, the addition of SUMO appears to cause Htt to accumulate in a non-aggregated or early aggregation state that is not visible by light microscopy. In light of recent studies showing that soluble oligomers that precede aggregates may be the toxic species, SUMO modification of Htt could be increasing the levels of these potentially toxic oligomers.

**Permanently SUMOylated Httex1p dramatically represses transcription.** Pathogenic processes in HD and other polyQ disorders appear to include repression of transcription by the mutant protein. Since SUMO-1 has been shown to play a role in transcriptional regulation through modification of transcription factors, we asked whether SUMOylation of Htt increases transcriptional repression. Because the fraction of Htt that is SUMOylated is quite low, we used permanently SUMOylated Htt to test the effects of modified Htt on transcriptional activity. We have previously shown that the

WAF1-pGL3 and the Multi Drug Resistance 1 (MDR1) gene promoters are repressed by expanded Htt (J. S. Steffan et al.,  
5 (2000) *Proc Natl Acad Sci U S A* **97**, 6763-68). When transfected into striatal cells, SUMO-97QP appears to dramatically repress both the MDR1 and WAF1-pGL3 promoters that are only modestly repressed by Httexlp 97QP (FIG. 3).  
10 These results are very similar to those in FIG. 2C, demonstrating dramatic repression of the CMV promoter by SUMO-97QP. These observations demonstrate that SUMO modification of Htt can substantially increase the suppressive effect of Htt on transcription, even with minimal SUMO-97QP protein  
15 levels present.

How might SUMOylation affect transcription? SUMOylation could cause increased nuclear or subnuclear localization of SUMOylated Httexlp, since SUMOylated proteins frequently  
20 localize to PML nuclear bodies that are implicated in transcriptional regulation. Modification of Htt by SUMO may increase the ability of Htt to be recruited to transcriptional repression complexes on chromatin. Alternatively, SUMOylation might alter cytoplasmic activity of Htt and either cause the  
25 release of a nuclear repressor that translocates to the nucleus or cause the cytoplasmic retention of proteins necessary to activate transcription, e.g. CBP.

**The CMV promoter is repressed by SUMO-97QP.** Control  
30 experiments to detect potential effects of these constructs on the expression of the CMV promoter driving the Htt transgene demonstrate that 97QP and 97QP K6,9,15R only modestly repress and rescue CMV expression respectively (Fig. 2C). Such minor  
35 effects would be expected if only a small fraction of Htt is SUMOylated as indicated in Fig. 1C. In contrast, SUMOylated 97QP (SUMO-97QP) significantly represses expression from the



CMV promoter. Notably, repression is largely abolished by mutation of the lysines even when SUMO is fused to the protein indicating that SUMO modification is necessary but not alone sufficient to affect repression. This observation suggests that two structural elements are required for transcriptional repression, namely SUMO modification and a structurally intact 1-17 amino acid domain. The repression of CMV by SUMO-97QP means that any potential accumulation of the SUMO-97QP protein is masked by the self-repression of the transgene (Fig. 2C). The steady state levels of SUMO-modified Htt (in the absence of direct fusion) are quite low, presumably due to competition with ubiquitination, limited SUMOylation and significant isopeptidase-mediated removal of SUMO; thus the permanent attachment of SUMO to all of the Htt protein reveals the dramatic stabilization that this modification can exert even when not in its normal side chain position(s). Chronic low level SUMOylation as seen in normal cells might be expected to contribute to progressive pathology of HD.

**Amino acids 1-17 of Htt comprise a cytoplasmic targeting sequence.** Since SUMO modification occurs in the first 17 amino acids of Htt, we asked whether this region influences subcellular localization of Htt since SUMO-1 modification can influence nuclear localization of proteins. We find that the first 17 amino acids of Htt can target proteins to the cytosol even when challenged with a strong Nuclear Localization Sequence (NLS). Specifically, when GFP is fused to the SV40NLS, its nuclear localization is greatly enhanced (FIG 4). However, the addition of the 1st 17 amino acids of Htt to the amino-terminus efficiently relocates this protein to the cytoplasm. Further, Httex1p, even when fused to an NLS, can be targeted to the cytosol when the first 17 amino acids of

Htt are present compared to complete nuclear localization when they are absent.

To further define the mechanism by which amino acids 1-17 of Htt accomplish cytosolic targeting, we assessed whether this sequence could act as an export receptor (CRM-1) dependent nuclear export signal (NES). Several large hydrophobic amino acids characteristic of an NES are indeed found in the 1st 17 amino acids of Htt. CRM-1 mediated export is abolished by Leptomycin B (LMB) treatment, therefore the effect of LMB on nuclear exclusion mediated by Htt 1-17 was tested. As expected, the nuclear exclusion of a control NES-GFP was efficiently reversed by LMB addition (FIG. 4) whereas the localization of GFP or NLS-GFP was unaffected. Surprisingly, LMB had no effect upon the nuclear exclusion of Htt 1-17-NLS-GFP, suggesting that Htt 1-17 either mediates a novel CRM-1 independent export function or it confers a cytoplasmic retention signal.

Interestingly, the two-amino acid spacing of the last two hydrophobic amino acids of Htt 1-17 (FX2L) are indeed in disagreement with the NES consensus sequence (LX(1-3)LX(2-3)LXL, L being a large hydrophobic residue). A possible mechanism whereby SUMO modification can enhance pathology is by masking of this cytoplasmic retention sequence. The demonstration that these 17 amino acids of Htt comprise a cytosolic retention sequence and also contain residues that can be SUMOylated raises the possibility that the SUMO modification described here may alter subcellular accumulation of Htt as well as modulate other properties of expanded Htt.

**The first 17 amino acids of Htt can target proteins to the cytosol even when challenged with a strong Nuclear Localization Sequence (NLS).** Since SUMO-1 modification can

influence nuclear localization of proteins and SUMO modification occurs on the first 17 amino acids of Htt, we asked whether this region influences subcellular localization. We find that the first 17 amino acids of Htt can target proteins to the cytosol even when challenged with a strong Nuclear Localization Sequence (NLS). This targeting may involve a novel CRM-1 independent export function or it may involve a cytoplasmic retention signal (FIG 4). In human patient brain tissue, mouse models and cell culture, mutant Htt protein is progressively localized from the cytoplasm to the nucleus. If nuclear localization of Htt is essential for HD pathogenesis, it is paradoxical that the pathogenic Htt fragment contains a cytoplasmic targeting signal. On the other hand, SUMO modification might preferentially mask this cytoplasmic retention signal in some cell types more than in others, allowing for different levels of nuclear localization and selective neuronal toxicity.

**Genetic reduction of SUMO activity in Drosophila reduces neurodegeneration in an HD fly model.** We next sought to genetically determine the relative contributions of SUMO and ubiquitin to pathogenesis. When mutant Httex1p (93QP) is expressed in all neurons of Drosophila, photoreceptor neurons are progressively lost and the integrity of the eye is compromised. However, when such animals are compared to sibs with reduced SUMOylation activity (i.e. heterozygous for a SUMO mutant, *smt3/+*), neurodegeneration is significantly reduced (FIG. 5A). In similar experiments, the level of ubiquitination activity was reduced and found to make pathology modestly worse (FIG. 5B). Thus SUMOylation makes pathology significantly worse while ubiquitination makes pathology modestly better.

Since the same lysines are targeted by both SUMOylation and ubiquitination, and since the global genetic reduction of both SUMO and ubiquitin can impact many cellular proteins and thereby indirectly affect the pathology of Htt, we sought to directly determine the influence of Htt modification on toxicity. Transgenic flies expressing Httex1p 97QP with or without the lysines mutated were compared. Mutation of the lysine residues dramatically reduces pathology (FIG. 5C). Similar experiments using the gmr-GAL4 driver that expresses the transgenes in all cells of the Drosophila compound eye confirmed that mutation of the lysine residues dramatically suppresses cytotoxicity (FIG. 5D), indicating that the availability of these lysines is essential to the pathogenic process.

Is the role of SUMOylation simply to prevent ubiquitination? If mutating the lysines served only to reduce ubiquitination, then pathology should worsen. Instead, the exact opposite is true, namely, pathology is dramatically reduced when the lysines are no longer available for post-translational modification. These data indicate that the inability to be SUMOylated has a more dramatic impact on pathology than the reduced ability to be ubiquitinated and degraded.

These observations demonstrate that Htt can be SUMOylated and suggest that SUMOylation can increase Htt accumulation, decrease aggregate formation and possibly increase toxic oligomers, potentially mask a cytoplasmic retention signal and increase nuclear repression of transcription. The impact of SUMOylation on HD pathogenesis *in vivo* is dramatic.

Decreasing expression of the SUMO-1 precursor, inhibiting SUMO-1 ligases, or increasing isopeptidase activity to remove SUMO-1 could each reduce the level of SUMOylated Htt in the cell and suppress pathogenesis. The E3 ligase specific for attachment of SUMO-1 to Htt presents a particularly attractive therapeutic target.

**Drug Screening Assays.** Transgenic animals, such as the Drosophila HD model described herein and in the references cited herein, may be used to identify compounds that reduce SUMOylation of mutant polyglutamine repeat proteins and which, therefore, are potentially useful in the treatment of HD and other polyQ-associated diseases. For example, fly HD models may be treated with various candidate compounds and the resulting effect, if any, on the rescue of neurodegeneration in the fly eye evaluated. The effect of candidate compounds on SUMOylation of Huntingtin and other polyglutamine repeat proteins may also be measured directly, using techniques known in the art. Preferably, the compounds screened are suitable for use in humans.

Drug screening assays in general suitable for use with transgenic animals are known. See, for example, U.S. Pats. Nos. 6,028,245 and 6,455,757. Various methods suitable for screening the efficacy of candidate compounds in reducing SUMOylation of glutamine repeat proteins are described herein or in the references cited herein. However, it will be understood by one of skill in the art that many other assays may also be used. Candidate compounds may be screened for their direct effect of the SUMOylation of polyglutamine repeat proteins or the screen can employ any phenomena associated with HD pathology that can be readily assessed in an animal model.

**Therapeutic Agents.** Once compounds have been identified in drug screening assays as eliminating or ameliorating the effects of HD pathologies, these compounds can be used as therapeutic agents, provided they are biocompatible with the animals, preferably humans, to whom they are administered.

The therapeutic agents of the present invention can be formulated into pharmaceutical compositions by combination with appropriate pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. Administration of the compounds can be administered in a variety of ways known in the art, as, for example, by oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intratracheal, etc., administration.

Depending upon the particular route of administration, a variety of pharmaceutically acceptable carriers, well known in the art can be used. These carriers include, but are not limited to, sugars, starches, cellulose and its derivatives, malt, gelatin, talc, calcium sulfate, vegetable oils, synthetic oils, polyols, alginic acid, phosphate buffered solutions, emulsifiers, isotonic saline, and pyrogen-free water. Preservatives and other additives can also be present. For example, antimicrobial, antioxidant, chelating agents, and inert gases can be added (see, generally, Remington's Pharmaceutical Sciences, 16th Edition, Mack, (1980)).

The concentration of therapeutically active compound in the formulation may vary from about 0.1-100 wt. %.

Those of skill will readily appreciate that dose levels can vary as a function of the specific therapeutic agents, the

severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given therapeutic agent are readily determinable by those of skill in the art by a variety of means. A preferred means is to measure the physiological potency of a given therapeutic agent.

The present inventors have shown that the Huntingtin protein can be SUMO-1 modified, that this modification stabilizes the protein, alters its aggregation and transcriptional repression properties and affects its pathogenic potential in a Drosophila model of HD.

In Huntington's disease (HD), pathogenic proteins with polyglutamine expansions accumulate. The present inventors have demonstrated that a pathogenic fragment of Huntingtin (Httex1p) can be modified either by SUMO-1 or by ubiquitin on identical lysines. In cultured cells, SUMOylation stabilizes Httex1p, affects transcriptional repression, and can alter the aggregation properties of Htt. In a Drosophila model of HD, SUMOylation exacerbates while ubiquitination relieves neurodegeneration. Further, mutations that prevent both SUMOylation and ubiquitination dramatically reduce pathology, indicating that the contribution of SUMOylation extends beyond simply preventing ubiquitination. Thus, treatments that modify SUMO activity should improve clinical outcomes in HD and other polyglutamine repeat diseases.

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While this invention has been described in detail with reference to a certain preferred embodiments, it should be  
15 appreciated that the present invention is not limited to those precise embodiments. Rather, in view of the present disclosure which describes the current best mode for practicing the invention, many modifications and variations  
20 would present themselves to those of skill in the art without departing from the scope and spirit of this invention. In particular, it is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, constructs, and reagents described  
25 as such may vary, as will be appreciated by one of skill in the art. The scope of the invention is, therefore, indicated by the following claims rather than by the foregoing description. All changes, modifications, and variations coming within the meaning and range of equivalency of the  
30 claims are to be considered within their scope.